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EFFECT OF POST-NATAL MATERNAL ENVIRONMENT ON FACTORS OF MOUSE EMOTIONALITY

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Effect of Post-Natal Maternal Environment on Factors of Mouse Emotionality", submitted by Franklin Wayne Poley in Partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

Pregnant female mice of two emotionally divergent strains, BALB/cJ and ST/bJ were ordered from Jackson Laboratory. These had previously been mated to males of their own strain. Litters of 3-4 pups, born at approximately the same time, were cross-fostered shortly after birth to mothers of the same strain or other strain. Active and passive stimulation of the young by the mother was then observed for 10 consecutive days. At 40 days of age a total of 41 offspring were administered a test battery consisting of 18 measures of emotionality. These measures were factor analyzed by Principal Axes factoring, Varimax rotation, to obtain 7 factors. The factors were then analyzed according to a 2 x 2 x 2 factorial design with main effects for post-natal maternal strain, offspring strain and sex.

Although strain differences in maternal stimulation of the young were not found to be significant, the post-natal environment of the young was proven to be of some importance as 1 main effect, 2 two-way interactions, and 2 three-way interactions involving the maternal variable were found to be significant. However, in terms of the total number of possible effects, post-natal maternal effects must be considered as the exception rather than the rule; and as yet there is no evidence that they can obliterate behavioral strain differences.

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INTRODUCTION

Research Objectives

The primary objectives of the research reported in this paper were to determine the following:

(a) Whether the two strains of mice, BALB/cJ and ST/bJ, which had been reported in an earlier study to be emotionally divergent, differ in their stimulation of offspring.

(b) Whether the post-natal maternal environment influences subsequent offspring emotionality.

(c) Whether there is a systematic relationship between maternal stimulation and offspring emotionality.

(d) Whether strain differences could be attributed to post-natal maternal effects.

(e) What the generality of the maternal effect might be by working with a number of orthogonal factors of emotional behaviour.

Maternal Stimulation and Offspring Behavior

Cross-fostering is a major means of studying the relationship between maternal stimulation of the young and subsequent offspring behavior. For example, Ressler (1962) used a 2 x 2 cross-fostering design with BALB/c and C57BL/10 mice and found a significant effect on the degree of stimulation of offspring both for pup strain and foster mother strain. That is, BALB/c mothers stimulate their offspring more than C57BL/10 mothers; and BALB/c offspring receive more stimulation than C57BL/10 offspring, regardless of the type of mother. Subsequently, Ressler (1963) studied the behavior of the offspring on a manipulation and visual exploration task. Subjects were placed in a darkened box such that door-pressing during the first 15 minutes was not followed by reinforcement, while door-pressing during the second 15 minute period was followed by 1 second of illumination. This gave a manipulation score for the first period and an exploration score for the second. Only the pup strain effect was significant for manipulation, but for visual exploration, the strain of foster parents was significant while all other main effects and interactions failed to reach significance. Subjects raised by BALB/c foster parents scored higher on visual exploration than subjects raised by C57BL/10 parents.

Reading (1966) worked with the same combination of strains and the same basic design. However, his dependent measures of offspring behaviour were more extensive, including open field activity, hole-in-wall latency, water-escape latency, barrier climbing and a composite defecation measure. For the hole-in-wall task, Reading found that BALB/c subjects raised by BALB/c mothers entered the goal compartment more rapidly than BALB/c subjects raised by C57BL/6 mothers. If this task is comparable to Ressler's visual exploration task it would seem that the greater maternal stimulation produced by BALB/c females enhances offspring curiosity or exploratory behaviour.

It would also appear from Reading's study that there is a direct relationship between degree of maternal stimulation and offspring emotionality. That is, BALB/c subjects were less active in the open field, and defecated more when raised by BALB/c mothers than by C57BL/6 mothers; C57BL/6 offspring had lower water-escape latencies and greater defecation when raised by BALB/c mothers.

Outside the findings cited above, there is little research directly related to the hypothesized relationship between maternal stimulation and offspring emotionality and curiosity. Seitz (1954) found that Wistar offspring from litters of 12 pups were more

emotional in the open field (less motility and greater defecation) than offspring from litters of 6 pups. Carlson (1961) also reported a direct relationship between litter size and emotionality with Wistar rats. Unfortunately, these findings do not agree with those of Reading (1966) since Seitz reported lower maternal stimulation scores for mothers with the larger litters. However, Seitz's maternal score was a composite measure derived from observations on 9 categories. These included nursing, reaction when cage door opened, reluctance to leave when approached, attempts to pick up the pups when litter was removed, searching behaviour when litter was absent, behaviour when litter returned, retrieving of pups, behaviour after cage door closed, and nest building. Most of these behaviours were mainly artifacts of the experimental situation, and consequently would not exert a great influence on the offspring throughout most of their pre-weaning environment. Thus, the research of Seitz (1954) and Carlson (1961) is not a definite contradiction to that of Reading.

Another area of research which is relevant involves subjecting mother animals to various stressful conditions and studying the effect on offspring behaviour. Stressful conditions have included the rotation of mothers between litter, electric shock, handling of female animals prior to mating, cross-fostering, blocked avoidance conditioning, and crowded living conditions. Unfortunately, this class

of research usually does not concern itself with what aspect of the changed mother-litter interaction might be responsible for any changes in offspring behaviour. Although adequate research on this question is not available, the assumption will be made that adverse stimulation of the mother leads to neglect or under-stimulation of the offspring. This is supported by casual observation of offspring neglect in the laboratory.

It is generally true in this area of research where an effect is found, that subjecting mother animals to adverse conditions results in increased emotionality in the offspring as a product of the post-natal maternal environment. Denenberg, Ottinger, and Stephens (1962) found that the rotation of mother Wistar rats every 24 hours, between litters born on the same day, and the post-natal shocking of mothers affected the body weight and mortality rate of the young. Rotating resulted in increased mortality and reduced body weight at 21 days; shocking resulted in reduced body weight at 62 days (but not at 21). This supports the suggestion that stressing the mother leads to neglect of the young. Shocking led to greater defecation in the open field; rotating led to increased defecation only when subjects were tested subsequent to an avoidance conditioning measure.

Denenberg and Whimbey (1963) handled female Wistar

rats in infancy for 20 days while control animals were not handled; at maturity these animals were bred and the offspring were cross-fostered to either a mother of the same background or a mother of a different background. Another condition allowed for non cross-fostering. At 50 days open field activity and defecation were observed. Considering the design as a 2 x 2 with post-natal effects for fostering or non-fostering, a significant interaction was found, with the non-handled, non-fostered group being more active than the other three. This offers some support for the position stated above. However, considering the design as a 2 x 2 with post-natal effects for fostering to handled or non-handled mothers, an interaction was observed with the non-handled natural mother, the handled foster mother group being the most active. That is, handling of the foster mother tended to reduce emotionality.

Thompson, Watson, and Charlesworth (1962) found a number of effects due to cross-fostering. In this study, unselected hooded rats were exposed to blocked avoidance conditioning during pregnancy and a 2 x 3 design was used. For open field activity at 130 and 140 days, activity was decreased for both cross-fostered experimental and control animals. However, there is some contradiction to the hypothesis of increased emotionality as cross-fostering tended to increase the difference between

experimental and control subjects by lowering activity in experimentals and raising it in controls. Also, fostering to the same type of mother produced increments in activity level for both experimental and control animals. For other dependent measures, however, the evidence is more definitely in favour of increased emotionality. Rearing by an experimental mother increased open field defecation, and "timidity" as measured by latency to leave the home cage and traverse a runway.

Hockman (1961) also worked with blocked avoidance conditioning during pregnancy in a 2 x 3 design. Pre-natal stress led to increased emotionality (less activity in the open field), but only if offspring were cross-fostered. Ader and Belfer (1962b) in a similar study found "some tendency" for animals reared by experimental mothers to defecate more in the open field than animals raised by control mothers. Keeley (1962) subjected pregnant albino mice to crowded living conditions. He found that emotionality decreased if subjects were not raised by their own mother. That is, latency to leave the home cage under conditions of hunger and non-hunger decreased.

Thus, although the evidence is not overwhelming, it would appear that this research contradicts the findings of Reading (1966). However, the apparent contradiction is based on an assumption which may not be valid

in all cases: that adverse treatment of the mother leads to understimulation of the young. Furthermore, effects may be dependent upon the strain of animal used as subject. Newell (1967) approached maternal stimulation by depriving young mice of the JK and BALB/c strains, of maternal care, 12 hours per day for 0, 4, 8, or 12 days during infancy. For emotionality as measured by open field defecation Newell found that maternal deprivation reduced emotionality in the BALB/c strain but increased emotionality in the JK strain. That is, the highly equivocal results in this area of research may be partly attributed to genetic differences of subjects used.

The generality of post-natal maternal effects is more readily assessed from the available research. Thompson and Olian (1961) state: "....we were not left with sufficient data to allow an analysis of post-natal effects by cross-fostering. However, previous work by the senior author (1957) has indicated that such influences are not of great importance." McIver and Jeffrey (1967) agree with this generalization in claiming: "There appears to be little support as yet for the assumption that maternal behaviour as measured to date is related to offspring emotionality." From an examination of the relevant literature, it would appear that the post-natal maternal environment may affect offspring behaviour, although it is not clear whether this is due to maternal behaviour itself since in every available study, maternal behaviour and nutrition are confounded.

In the studies reported above only a small portion of the total number of main effects and interactions involving the post-natal maternal environment are statistically significant. Furthermore, a number of studies have reported no post-natal maternal effects. Thus, Broadhurst and Levine (1963) found that subjects from litters of 2-4 pups did not differ from those of 5-9 pups for open field defecation and activity.

Thompson and Sontag (1956) in a study of the effect of audiogenic seizures in pregnant rats on the behaviour of the offspring found that cross-fostering produced no significant effects for water-maze learning, general activity, or body weight. McIver and Jeffrey (1967) found differences in maternal behaviour between Long Evans, Wistar and Sprague-Dawley rats. However, no systematic relationship between maternal behaviour and offspring open field activity was found.

Maternal Effects and Research Design

Although it is true that post-natal maternal environment affects offspring behaviour in a minority of cases, it is not true that post-natal maternal environment is inconsequential. There are two areas of research where control for this environmental effect would be required for completely unambiguous results. First of all, in genetic mammalian research, strain differences themselves may not be evidence of genetic differences but rather differences in maternal environment. Thus, the appropriate control would involve a reciprocal cross procedure. For example, Collins (1964) used a diallel cross to study the inheritance of avoidance conditioning in inbred mice. Another area of research is that which studies the effect of various agents and experiences to which a pregnant animal may be subjected, on offspring behaviour. Without the proper control, which in this case is cross-fostering, results will be ambiguous as to whether an influence is exerted pre-natally or post-natally.

SUBJECTS AND DESIGN

Pregnant female mice were ordered from the Jackson Laboratory. These mice were approximately 2 weeks pregnant (mice typically give birth at 3 weeks) on arrival. Shipping itself was not expected to be an important source of confounding since mice were transported by air and spent only approximately 24 hours in transit. Females were either of the ST/bJ or BALB/cJ inbred strains, no more than 10 generations removed from a common ancestral pair. The common ancestral parents are the product of more than 20 generations of brother-sister matings (63 in the case of ST/bJ mice and 81 for BALB/cJ mice as of August 1961, according to the 1962 Jackson Laboratory Handbook). Females had been mated at Jackson Laboratory to males of their own strain.

The basic design in this research was a 2 x 2 factorial design in which main effects were due to post-natal maternal strain or pup strain. Three female mice and offspring were assigned to each condition to yield a total of 12 litters. All litters used were cross-fostered after birth to a mother of the same strain or other strain. Final analysis was made according to a 2 x 2 x 2 design with sex added as a condition. The number of subjects in each of the 8 cells is given in Table 18. 42 subjects were available at the beginning of testing. Since one animal died, only 41 offspring

completed the final test battery.

Choice of strains and measures was based upon a previous factor analytic study of emotionality in inbred mice. In this study (Royce and Carran, 1967, unpublished data) BALB/cJ and ST/bJ mice were found to be the two most divergent strains, differing significantly on 3 factors (see Figure 4). These factors with the measures and loadings upon which the selection was made are as follows: Factor II, Underwater Swimming Latency to Traverse .60, Pipe Urination .53, Straightaway Defecation .47; Factor V, Open Field Activity -.53, Avoidance Conditioning -.41, Pipe Urination -.35; Factor XI, Open Field Urination .51, Straightaway Urination .38, Activity Wheels A.M. .29. All measures were used with the exception of Underwater Swimming. Furthermore, 13 other observations appropriate to the apparatus employed were taken to give a total of 18 measures.

ANALYSIS

The analysis of maternal stimulation was conducted according to a standard analysis of variance with main effects for foster mother strain and offspring strain, and a trend for the 10 days of observation.

For the 18 dependent variables which resulted from the final test battery administered to the offspring, the first stage in the analysis involved a new factor analysis of the data. Those factors mentioned previously, upon which the choice of strains was based, were not employed in this study. Invariance was not expected for the following major reasons: (1) certain apparatus changes, notably in illumination, had been conducted since the original study. (2) The original study was based upon 10 strains of mice whereas only 2 were used here. (3) The test battery employed in this study was a considerably abbreviated version of the original battery. Thus, an orthogonal method of factoring was chosen for convenience.

Thus, the 18 dependent variables were factor analyzed by Principal Axes factoring (H. H. Harman, Chapter 8), with Varimax rotation (H. H. Harman, pp. 304-313). Factoring was terminated when eigenvalues became less than 1.00, which yielded 7 factors. From this, factor scores were calculated (H. H. Harman, pp. 345-354). Factor

scores were computed according to the formula: $F = A R^{-1} Z$ where A is the n x r factor structure matrix, R^{-1} is the inverse of an n x n correlation matrix (original data), Z is the N x n matrix of original scores (standardized), and F is the r x N matrix of factors, given: N = # subjects, n = # variables, r = # factors. Factor scores were then employed in a series of 7 analyses of variances (one for each factor) according to a 2 x 2 x 2 design and General Linear Model with a Least-Squares Solution for dealing with unequal cell frequencies (Winer, 1962, pp. 224-227). Here, main effects are due to mother strain, offspring strain and offspring sex. Maternal stimulation was analyzed according to a 2 x 2 x 10 design with main effects for mother strain, offspring strain and days of observation.

TESTS AND MEASURES[1]

Maternal Stimulation

Mother and litter were housed in standard sized, translucent plastic nesting boxes ($7\frac{1}{4}$" wide x $7\frac{1}{4}$" deep x 11" long) containing sawdust and shavings. Food and water were provided on an ad-lib basis. Nesting boxes were located behind a cheese-cloth screen for observation purposes. Two mercury switches were available, connected to 60 minute Gra-Lab timers in order to record the mother-litter interaction. The first timer was used to record the time devoted to "passive stimulation" of the young by the mother. Passive stimulation was defined as the time spent on the nest in contact with at least two pups. The second timer was used to record "active stimulation" which was defined by actual grooming, nudging, carrying of the pups and related activities whether these gave the appearance of being intentional or unintentional. Active stimulation was recorded as long as one pup or more was being stimulated in the manner described.

Avoidance Conditioning

The Avoidance Conditioning apparatus used has been described more fully elsewhere (Yeudall, Royce and De Leeuw, 1968). In summary, it consists of a shuttle box ($3\frac{1}{2}$" wide x $1^{5/8}$" high x $15\frac{1}{2}$" long) which is mounted inside an insulated chamber (13" wide x 12" high x 21" long) with a

one-way mirror in the door. Photo-electric cells are mounted in such a way as to focus across the mid-line of the shuttle box. One speaker is located at each end of the shuttle box. The chamber is illuminated by a 6 watt fluorescent light and is ventilated by a small fan. The control unit located in a separate room allows for an adjustable CS-UCS and rest period. Inter trial activity is recorded as the animal breaks the photo-cell beam during the rest period. For the purpose of this research CS was set at 3 seconds, UCS at 3.5 seconds, rest period at 120 seconds, and CS intensity at 400 volts. Two measures were taken from this apparatus: correct number of avoidances and inter trial activity. These are listed in Table 6 with their means and variances as variable 1 Avoidance Conditioning, and variable 2 Inter Trial Activity A. C.

Activity Wheel

Activity wheels, constructed by Acme Metal Products, Inc., Chicago, consisted of an aluminum drum, 6 inches in diameter with a 2 inch wide band for running, perforated with .125 inch holes. A living cage ($4\frac{1}{2}$" x 3" x 3") attached to the structure allowed free access to the wheel. Revolutions in either direction activated a cumulative recorder. A.M. and P.M. measures of activity were recorded (variables 3 and 4 in Table 6) according to the light cycle under which all animals were maintained, whereby lights were turned on at 9:30 A.M. and off at 9:30 P.M.

Open Field

The Open Field used was a flat white masonite circle, 4 feet in diameter, divided by concentric circles painted with a .4 centimeter flat black lines. The outermost region, bounded by a 12 inch high sheet metal wall and a 34 inch circle on the other side was divided into 16 equal sized areas by lines radiating outward. The next region, bounded on the inner side by a 20 inch circle was also divided into 16 areas. Next to this was an area bounded by a 6 inch circle and divided into 8 equal portions. The centre of the field with the 6 inch diameter was undivided. The entire field was covered with a transparent plexiglass sheet. The field was housed in a structure of $\frac{3}{8}$" plywood (64" long x 52" wide x 60" high) with a one-way glass window ($17\frac{1}{4}$ x $14\frac{1}{4}$") on one side, and a door on an adjacent side for entry. Four inches from the window a nylon cord led through the wall to a steel bracket 46 inches above the outermost region of the field. A plexiglass clamp was attached to the end of the cord; the clamp could be fastened to a transparent plexiglass starting box ($4\frac{1}{4}$" x $1\frac{3}{4}$" x $2\frac{1}{4}$"). From the ceiling of the structure housing the open field, a bank of fluorescent lamps behind translucent paper provided uniform illumination at 130 foot candles as recorded by a Weston Illumination Meter.

Prior to each measure, the field was washed with water containing R2L disinfectant and allowed to dry.

The subject was removed from its home cage and placed in the portable starting compartment. The starting compartment was then lowered to the center of the portion of an outer annulus of the open field. The previously described clamp was attached to the compartment, the sliding bottom was removed, and the experimenter quietly left the housing, closing the door behind. From the front of the housing, before the one-way window, the nylon cord was raised at the same time that a stop-watch was started and a switch attached to a Gra-Lab timer. When subject had placed four paws in the next section of the open field the timer was stopped. During two minutes time the number of sections traversed by subject were counted according to a criterion of all four paws across a demarcation line.

At the end of the two minutes subject was returned to its home cage. Thus, the following measures were involved: (a) latency to leave the starting section in seconds (variable 5 in Table 6), (b) number of lines traversed (variable 6 in Table 6), (c) presence or absence of urination (variable 7 in Table 6), (d) number of boluses of defecation (variable 8 in Table 6).

Straightaway

Straightaway consisted of a runway (50" long x 1.5" wide) elevated 31 inches from the floor. The runway was divided by a .125 inch black stripes into 11 interior sections each 3.75 inches long and two 2.875 inch sections

at either end. Covering the runway was a transparent plexiglass strip $\frac{1}{8}$" thick with attached wire mesh of $\frac{1}{16}$" squares. The apparatus was housed in a box (62" long x 14" wide x 56" high) of $\frac{3}{8}$" plywood with a large door at front and one-way glass (36" long x $2\frac{1}{2}$" wide) for observation. A smaller hinged door was located in the center of the housing for placing subject on the center division of the straightaway. Illumination read from the surface of the runway was 20 foot candles. Lighting was provided by pink, fluorescent lights behind flashed, opal glass. The four measures taken were the same as those for the open field. Variables 9 to 12 are included in Table 6.

Pipe

The Stove Pipe apparatus consisted of two white opaque goal boxes ($6\frac{1}{4}$" square x 4" deep) connected by a gray plastic tube 24 inches long with $2\frac{1}{8}$ inch inside diameter. Each goal box had a hinged, transparent lid perforated with air holes. On one side of each goal box a circular opening covered by a sliding opaque door led to the pipe. Housing for the pipe was again $\frac{3}{8}$" plywood box (30" wide x 43" long x 22" high) with a hinged door at the front, and a 5x5 inch one-way window mounted near each end. Nine inches from either ends cords connected to the side doors of the pipe led through the housing. Once again illumination at 20 candles was

provided by a bank of pink, fluorescent lights behind flashed, opal glass. Measures taken from this apparatus were as follows: (a) urination (variable 13), (b) defecation (variable 14), (c) latency to enter the pipe from the goal box in seconds on trial 1 (variable 15), (d) latency to enter the pipe in seconds on trials 2, 3 and 4 (variable 16), (e) latency to emerge from the pipe in seconds into the opposite goal box on trial 1 (variable 17), and (f) latency to emerge from the pipe in seconds into the opposite goal box on trials 2, 3 and 4 (variable 18).

PROCEDURE

Upon arrival from Jackson Laboratory, pregnant females of the two strains were placed in standard sized nesting boxes with ad-lib food, water, shavings, and sawdust. All animals were maintained for their lifetime on a light cycle with complete darkness from 9:30 P.M. to 9:30 A.M. The day of birth of each litter was recorded and counted as Day 1. If another litter of mice was born on either Day 1, Day 2, or Day 3 of the first litter these two litters were cross-fostered on Day 4. Cross-fostering consisted of removing pups from the nest with clean foreceps and placing them in a petrie dish for a few minutes until they could be switched to the new mother. Litters consisting of three or four pups were used; larger litters were pruned to four. If pups died before weaning the surviving litter mates were kept for testing provided at least two pups survived to weaning in each litter. On Day 5 observation of the mother-litter interaction began and continued for ten successive days. On each of these days the time of observation was dictated by convenience, but the average time of observation for each of the four conditions was approximately the same. Inter-observer reliability coefficients were calculated by having one of the laboratory personnel[2] read the description of active and passive stimulation, then make observations for these two measures

on a litter of mice for a five minute period. Litters for this purpose were randomly chosen from mice bred in the laboratory; 10 three-minute observation periods were used during which the experimenter also took observations on the litter chosen. A Pearson product moment correlation coefficient was calculated for these ten periods. Two laboratory personnel participated in these ratings. In each case reliability coefficients were calculated between the ratings of the experimenter and the ratings of each of the personnel.

Each observation period in the study itself was 15 minutes in duration; subjects were in no way disturbed during this period. Cages were cleaned only when absolutely necessary, as for example, when a water bottle accidentally drained. When each litter was 21 days of age (counting the day of birth as Day 1) it was weaned by removing the mother and leaving the pups in the nesting box in which they had been born.

At 40 days of age subjects were weighed, sexed, and then placed individually in wire cages (4" x 4" x 8") on the laboratory racks. All measures taken subsequent to this involved the use of white noise. The white noise speaker was set at approximately 30 inches from the apparatus with intensity of 85 dB. Also on Day 40 Avoidance Conditioning testing began with subjects given a five minute period of adaptation in the apparatus

without CS or UCS presentations. On Day 41, Avoidance Conditioning began with a pre-conditioning phase during which subjects received 5 presentations of CS without UCS (each presentation separated by 120 seconds), and 5 presentations of UCS without CS (also separated by 120 seconds). Immediately following this pre-conditioning phase, conditioning itself commenced with 25 trials of CS-UCS presentations. If subject ran to the opposite side of the shuttle box during the 3 second CS period, CS was terminated and a correct trial was recorded; if subject failed to make the avoidance response during this period a 3.5 second shock presentation signalled an error. Even if subject made a correct avoidance during the CS period it was possible for him to make an error on this trial by immediately running back into shock. A rest period between each CS-UCS presentation of 120 seconds was allowed during which inter trial activity was automatically recorded.

At the end of 25 trials the apparatus automatically stopped; correct avoidances and inter trial activity were recorded by the experimenter. On Days 42 and 43 subjects received 25 trials of CS-UCS presentations without pre-conditioning. Thus, a conditioning score for a total of 75 trials was obtained for each; also the inter trial activity for these 75 trials. Avoidance Conditioning was completed for each subject no later than 5:30 P.M. on

Day 43, whereupon subject was placed in the Activity Wheel compartment, but prevented from running in the wheel by a door until 9:30 P.M. of that evening. At 9:30 P.M. the number of counts on the Activity Wheel recorded were noted and the door to the running surface of the wheel was opened. Three additional readings of this counter were taken at 9:30 A.M., 9:30 P.M. of the next day, and at 9:30 A.M. of Day 45. At 9:30 A.M. on Day 45 each subject was removed from the Activity Wheel and returned to his individual cage on the rack.

On Day 46 subjects were tested in the Open Field and Straightaway apparatus. Open Field testing began at approximately 4:00 P.M. and Straightaway approximately 1 hour after the completion of the Open Field test. The four measures taken in each apparatus have already been described. Between the testing of subjects in the Open Field the field was washed with water containing R2L disinfectant, and allowed to dry. This was not necessary for Straightaway since a different plexiglass strip was placed over the runway for each subject. Day 47 was a repetition of the procedure of Day 46.

At 9:30 A.M. of the next day subjects were deprived of food for a 12 hour period following which the Stove Pipe measure was begun.

For the Stove Pipe measure subject was placed in the left goal box and two 5 milligram food pellets were placed

in the open goal box. The door to the pipe was closed before subject was placed in the compartment; subject was allowed 2 minutes to adapt to its new surroundings. As soon as the subject was observed sniffing the door leading to the Stove Pipe following this 2 minute period, this entrance door was opened by a draw string which automatically closed the exit door leading from the Stove Pipe to the other goal box. At the same time that the door was opened a timer started. If subject immediately turned away from the entrance door as it was opened the timer was stopped and the trial was started over again since it was not clear whether subject had in fact observed that the door was opened. If subject continued to sniff in the vicinity of the door, no matter how brief the period, the trial was continued. As soon as the subject placed all four paws inside the Stove Pipe, the timer was stopped which automatically started another timer, and the draw-string was pulled to close the door behind subject, being careful not to pull the subject's tail in the doorway. Pulling the draw-string also opened the exit door from the pipe. When the animal had emerged, the door was closed behind him, and the second timer was stopped. When subject had consumed the two food pellets this procedure was repeated in the opposite direction. Urination and defecation measures were not taken until subject had completed

both trials. After completion of the measure subjects were returned to ad-lib food and water in their individual cages until 9:30 A.M. of the next morning when this procedure was repeated to give the final observations for the test battery.

RESULTS

Three major phases were involved in the analysis of the data. The first phase was related to the post-natal stimulation of offspring by mother. The second phase pertained to the factor analysis of the test battery administered to the offspring. The third phase involved an analysis of variance of the obtained factor scores to determine maternal effects. For reference in this section and in the series of tables which present various analyses, "A" will be used to refer to post-natal mother strain; "B" will be used to refer to pup strain; "C" will refer to sex; and "D" will refer to days.

Maternal Stimulation

A number of fosterings were unsuccessful in that the fostered pups were eaten or neglected by the mother. Of the switched litters which were initially successful in that observations of the mother-litter interaction were begun, some pups did not survive to weaning. The number of pups which did not survive to weaning in these cases are recorded in Table 1. Here, $P < .005$ (Fisher's Test), indicating that offspring of the same strain as the mother receive preferential treatment.

Since three days were allowed between births for fostering to be done, offspring ages and the number of

days post-parturition of the foster mother, of necessity varied somewhat. These ages are given in Table 3 for each litter used in the final test battery. Mean ages of offspring compare favorably, being 2.3, 3.3, 3.7, and 3.3 for each of the four cells in the basic 2 x 2 design. Mean number of days post-parturition of the foster mothers for these pups were 3.7, 3.7, 3.0 and 2.7.

Analyses of the observations of active and passive stimulation of the pups by foster mothers are given in Tables 4 and 5. None of the main effects or interactions were significant. However, the sample size for this analysis was very small with only 12 litters and consequently only 12 mothers used in the study. Thus, mean active and passive stimulation are presented according to the four conditions of rearing, in Table 2, in order to suggest where effects might be found if a larger sample of mothers were used. From Table 2, a main effect is suggested with ST/bJ mothers stimulating their offspring more, along both dimensions, than BALB/cJ mothers. The mean number of seconds of active stimulation for ST/bJ mothers is 337.5 and only 169.5 for BALB/cJ mothers; the mean number of seconds of passive stimulation is 4258 for ST/bJ mothers and 3396 for BALB/cJ mothers.

Reliability coefficients were favorable, being approximately .80 between the experimenter and each of two laboratory personnel for active stimulation and

approximately .90 for passive stimulation.

Factor Analysis

The 18 measures of the test battery administered to offspring beginning at 40 days of age are listed in Table 6 with the overall means and variances. Principal Axes factoring with Varimax rotation yielded 7 factors. The intercorrelation matrix from which the factors were derived is given in Table 7 while Table 8 gives factor loadings, communalities and sum of squared loadings of factors.

Interpretation of Factors[3,4]

Factor I Five measures have loadings of .30 or higher on this factor. They are:

01	Avoidance Conditioning	.84
12	Straightaway Boluses	.66
07	Open Field Urination	.64
18	Pipe Latency 2 3 4 Emerge	-.57
03	Activity Wheels A.M.	-.57

This factor is interpreted as Primary Approach Avoidance since measure 01 involves avoidance of pain and measure 18 involves approaching food under conditions of deprivation. Also, measure 03, A.M. activity, would be affected by whether the animal remains in the home cage where food and water are available or runs in the wheel.

Factor II Six measures have loadings of .30 or higher on this factor. They are:

10	Straightaway Activity	.85
06	Open Field Activity	.70
15	Pipe Latency 1 Enter	.53
05	Open Field Latency	-.37
14	Pipe Boluses	.36
16	Pipe Latency 2 3 4 Enter	.32

This factor is interpreted as <u>Curiosity in Open Light</u> as indicated by the correspondence between high activity in the Open Field and Straightaway and a low latency to leave the starting section of the Open Field. Furthermore, these activity measures correlate positively with two measures of latency to enter the Pipe (15 and 16). The starting box of the pipe is a highly illuminated field as is true of the Open Field and Straightaway although the field for the Pipe is smaller. High activity in the starting box of the Pipe would compete with tendencies to enter the Pipe and account for corresponding high latencies. To distinguish this factor from Factor III it should be noted that gross bodily activity is involved.

<u>Factor III</u> Five measures have loadings of .30 or higher on this factor. They are:

05	Open Field Latency	-.71
08	Open Field Boluses	.70
11	Straightaway Urination	-.48
17	Pipe Latency 1 Emerge	-.37
13	Pipe Urination	.34

This factor is interpreted as <u>Emotional Reactivity to Open Light</u>. Three of the measures loading on Factor III involve elimination and the other two involve latencies to move in an illuminated field. Thus, this is considered

as a fear or freezing factor as opposed to Factor II which, although it involves a similar setting, results in gross motor activity.

Factor IV Five measures have loadings of .30 or higher on this factor. They are:

17	Pipe Latency 1 Emerge	.84
18	Pipe Latency 2 3 4 Emerge	.55
13	Pipe Urination	.49
09	Straightaway Latency	.39
03	Activity Wheel A.M.	.33

Factor IV is interpreted as Light-Darkness Preference. A high latency to emerge from the Pipe suggests a preference for darkness (measures 17 and 18) while a high urination scores on measure 13 indicates an aversion to light (Urination and Defecation were always found in the goal boxes of the Pipe, never in the connecting tube.) A high straightaway latency may be thought of in a similar manner and nocturnal activity (measure 03) would indicate a preference for the dark.

Factor V Four measures have loadings of .30 or higher on this factor. They are:

04	Activity Wheel P.M.	.84
07	Open Field Urination	.49
13	Pipe Urination	-.49
09	Straightaway Latency	.46

This factor is interpreted as Diurnal Activity based on the high loading of measure 04. Also, this is the only factor to which measure 04 does contribute a loading above .30.

<u>Factor VI</u> Four measures have loadings of .30 or higher on this factor. They are:

14	Pipe Boluses	.70
16	Pipe Latency 2 3 4 Enter	-.65
09	Straightaway Latency	.35
12	Straightaway Boluses	.32

This factor is interpreted as <u>Timidity.</u> The Pipe measure is commonly referred to in the comparative-developmental literature as a measure of "timidity", suggesting hesitation to approach food under conditions of hunger (measure 16) and emotional reactivity in the situation (measure 14). Straightaway latency and defecation could also be considered as indicators of timidity in the sense of emotional reactivity.

<u>Factor VII</u> Six measures have loadings of .30 or higher on Factor VII. They are:

02	Inter Trial Activity A.C.	.84
18	Pipe Latency 2 3 4 Emerge	.56
03	Activity Wheel A.M.	.41
09	Straightaway Latency	-.41
11	Straightaway Urination	-.38
13	Pipe Urination	-.31

Factor VII is interpreted as <u>Emotional Reactivity to Conditions of Primary Drive.</u> It is similar to Factor I in that conditions of food deprivation and electric shock are involved. However, there is a greater element of non-goal directed activities here. For example, measure 02, inter trial activity in the avoidance conditioning situation, loads on this factor, Also, two urination measures load on the factor.

Analysis of Maternal Effects

Phase 3 of the analysis involved obtaining factor scores for subjects (given in Table 9) and applying a 2 x 2 x 2 analysis of variance to these factor scores. The results of the analysis of variance are given in Table 10 to 16 inclusive. Table 17 gives the results of the same analysis for offspring weights, taken at 40 days.

Of the 49 F ratios calculated for the 7 factors, 7 F's are significant. Five of these involve post-natal maternal stimulation in some fashion including one main effect, two two-way interactions and two three-way interactions.

Analysis of Variance

Factor I, interpreted as Primary Approach-Avoidance, yields two significant interactions involving the maternal variable. For the A x C (post-natal maternal strain x sex) interaction, males score lower on Factor I than females when raised by an ST/bJ foster mother, but the sex difference is reversed for a BALB/cJ foster mother. The A x B x C interaction is given in Figure I (B refers to pup strain). In this case the factor score for males is higher than that for females for BALB/cJ offspring while no sex differences appear for ST/bJ offspring when subjects are raised by a BALB/cJ foster mother. On the other hand, with ST/bJ foster mothers, BALB/cJ males

score lower than BALB/cJ females while there are negligible sex differences for ST/bJ offspring.

There is also a significant A x B x C interaction for Factor IV, Light-Darkness Preference. Here, ST/bJ males score somewhat higher than females while BALB/cJ males score considerably higher than females when subjects are raised by a BALB/cJ mother. For rearing by an ST/bJ mother, the sex difference holds for ST/bJ offspring but is reversed for BALB/cJ offspring. (See Fig. 3)

Figure 2 describes what is perhaps the most interesting interaction: the A x B interaction for Factor VI, interpreted as Timidity. In this case, the strain difference between ST/bJ mice and BALB/cJ mice is completely reversed, depending upon the post-natal maternal environment. Thus, ST/bJ's score higher on this factor than BALB/cJ's when raised by an ST/bJ foster mother, whereas ST/bJ's score lower when subjects are raised by a BALB/cJ foster mother.

An additional maternal effect involves the A main effect for Factor III, Emotional Reactivity to Open Light. ST/bJ foster mothers produce offspring with higher scores on this factor than do BALB/cJ foster mothers. ($\bar{X} = 53.29$ vs $\bar{X} = 46.32$).

The two additional significant F ratios involve a B (pup strain) main effect for Factor I, and a C (sex) main effect for Factor IV. BALB/cJ pups score higher on

Factor I (Primary Approach-Avoidance) than ST/bJ pups ($\bar{X}$ = 57.95 vs $\bar{X}$ = 38.59), and males score higher on Factor IV (Light-Darkness Preference) than females ($\bar{X}$ = 53.40 vs $\bar{X}$ = 47.06). Significant effects were also found for weight (Table 17) with ST/bJ offspring weighing more than BALB/cJ and males weighing more than females.

DISCUSSION

The first question posed in the introduction to this paper was whether the two emotionally divergent strains, BALB/cJ and ST/bJ, differ in their stimulation of offspring. Some difference is suggested by the data; however, this difference does not reach the level of significance ($p > .05$). Moreover, the suggested difference is in the direction of greater stimulation administered by ST/bJ mothers, along both dimensions of stimulation measured.

The answer to the second question (whether post-natal maternal environment influences offspring emotionality) is a definite affirmative. The five significant effects involving the maternal variable were explained earlier. However, only one of these corresponds to the suggested main effect in maternal stimulation. This is the A main effect for Factor III, Emotional Reactivity to Open Light. For this factor, a high score indicates low reactivity or fear since there is a preponderence of negative loadings. Thus, in answer to the third question, the suggested inverse relationship between maternal stimulation and offspring emotionality is in disagreement with the findings of Reading (1966). The exact origin of the observed effects, however, is not revealed. It could possibly be found in some aspect of maternal stimulation not observed or in nutritional factors. In Table 17, the

A main effect is significant at the .10 level which suggests a maternal effect on offspring weight. However, this could be a product of maternal stimulation rather than nutrition, or as well as nutrition. Thus, a strong systematic relationship between maternal stimulation and offspring behavior was not found.

That strain differences may be confounded by maternal effects is suggested by the A x B interaction for Factor VI, Timidity, where a complete reversal of the strain differences is found, depending on the post-natal maternal environment. In other words, subjects are less timid when raised by a mother of the other strain. In addition, the A x C interaction for Factor I, Primary Approach-Avoidance, is subjected to the same reversal. That is, a sex difference which might otherwise be attributed to a genetic origin is completely reversed, depending on the strain of mother. However, Factor I was the only factor where a main effect for offspring strain was found and there is no suggestion here that the strain difference could be attributed to a maternal effect. This is in complete agreement with the findings of Ressler (1963) and Reading (1966). Although these writers have stressed the importance of their maternal effects, it is quite clear that there is no evidence offered by any of these studies to refute the notion of genetically determined strain differences. What has been demonstrated is that both genotype and maternal environment may affect

behavior independently or in interaction.

The magnitude or generality of the post-natal maternal effect is best determined by examining the analysis of variance for the 7 orthogonal factors. Of 7 main effects involving the maternal variable, only 1 was significant; and of 28 interactions involving the maternal variable, only 4 were significant. This finding agrees with the survey of the relevant literature in which it was found that post-natal maternal effects are a possibility, and perhaps even an important possibility, but that they are not a general occurence in comparative-developmental research.

FOOTNOTES

1. All apparatus, with the exception of that used for observing maternal stimulation was provided and developed by the laboratory of Dr. J. R. Royce, through Canadian Public Health Research Grant DPH 33, 608-7-82 and a National Research Council of Canada Grant APT-105. A recent paper by Royce, Carran, and Howarth (1967) was of considerable assistance in describing the apparatus although a number of changes have since been conducted.

2. The author would like to thank Pat Yeudall and Bob Barton for assisting with inter-observer ratings of the mother-litter interaction.

3. Two points with respect to the factor analysis should be mentioned here, although they are peripheral to the main purpose of the study. The first is that experimental dependence of the factors may be a slight problem since four measures of Pipe latency were recorded. The second is that although complete factor invariance should not be expected for the reasons mentioned earlier, Factor II of this study does resemble Factors II and VI of an earlier study by Royce and Yeudall (1967), and Factor A of Royce, Carran, and Howarth (1967), where orthogonal solutions were used in both studies.

4. All factors interpretations are based upon the author's observations of the subjects' behavior, and are meant to be of a tentative nature.

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TABLE 1

NUMBER OF SUBJECTS NOT SURVIVING TO WEANING ACCORDING TO THE FOUR CONDITIONS OF REARING

Post-Natal Maternal Strain	Pup Strain: BALB/cJ	Pup Strain: ST/bJ
ST/bJ	8	1
BALB/cJ	0	6

TABLE 2

MEAN NUMBER OF SECONDS OF ACTIVE STIMULATION OVER 10 DAYS FOR EACH CONDITION OF REARING (PER LITTER)

Post-Natal Maternal Strain	Pup Strain: BALB/cJ	Pup Strain: ST/bJ
ST/bJ	344	331
BALB/cJ	221	118

MEAN NUMBER OF SECONDS OF PASSIVE STIMULATION OVER 10 DAYS FOR EACH CONDITION OF REARING (PER LITTER)

Post-Natal Maternal Strain	Pup Strain: BALB/cJ	Pup Strain: ST/bJ
ST/bJ	4320	4196
BALB/cJ	3557	3235

TABLE 3

AGE OF PUPS AND NUMBER OF DAYS
POST-PARTURITION OF FOSTER MOTHER
AT THE TIME OF FOSTERING

Pup Strain	Mother Strain	Age of Pups at Fostering (days)	No. Days Post-parturition of Mother at Fostering
BALB/cJ	ST/bJ	2 3 2	3 4 4
BALB/cJ	BALB/cJ	4 2 4	4 4 3
ST/bJ	ST/bJ	3 4 4	4 3 2
ST/bJ	BALB/cJ	2 4 4	2 3 3

TABLE 4

ANALYSIS OF VARIANCE
FOR ACTIVE STIMULATION

Source	Sum of Squares	d.f.	Mean Square	F
A	6855.41	1	6855.41	0.82
B	1680.01	1	1680.01	0.20
A x B	1159.41	1	1159.41	0.14
S (AB)	67129.80	8	8391.22	
D	31222.24	9	3469.14	1.18
A x D	23028.18	9	2558.69	0.87
B x D	17310.24	9	1923.36	0.65
A x B x D	29697.18	9	3299.69	1.12
ERROR	211764.87	72	2941.18	

TABLE 5

ANALYSIS OF VARIANCE
FOR PASSIVE STIMULATION

Source	Sum of Squares	d.f.	Mean Square	F
A	218197.41	1	218197.41	0.34
B	17400.21	1	17400.21	0.03
A x B	2403.08	1	2403.08	0.00
S (AB)	5165680.60	8	645710.00	
D	1956724.71	9	217413.75	1.83
A x D	1178197.84	9	130910.75	1.10
B x D	1680219.04	9	186691.00	1.58
A x B x D	1686882.84	9	187431.31	1.58
ERROR	8543152.07	72	118654.88	

TABLE 6

MEANS AND VARIANCES OF THE 18 MEASURES INCLUDED IN THE TEST BATTERY

	Variable	Mean	Variance
1	Avoidance Conditioning	19.93	167.87
2	Inter Trial Activity A. C.	208.96	16034.50
3	Activity Wheel A.M.	6894.46	17893776.00
4	Activity Wheel P.M.	1268.66	1510291.00
5	Open Field Latency	10.46	12.21
6	Open Field Activity	51.00	304.20
7	Open Field Urination	1.15	0.58
8	Open Field Boluses	4.88	7.31
9	Straightaway Latency	8.20	10.27
10	Straightaway Activity	101.49	976.36
11	Straightaway Urination	0.59	0.50
12	Straightaway Boluses	2.20	4.91
13	Pipe Urination	0.63	0.49
14	Pipe Boluses	0.95	3.20
15	Pipe Latency 1 Enter	24.05	857.00
16	Pipe Latency 2 3 4 Enter	15.07	268.37
17	Pipe Latency 1 Emerge	19.56	143.45
18	Pipe Latency 2 3 4 Emerge	16.76	111.99

TABLE 7

INTERCORRELATION MATRIX FOR THE 18 DEPENDENT VARIABLES

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.00																	
2	-0.04	1.00																
3	-0.37	0.28	1.00															
4	-0.04	0.01	0.22	1.00														
5	0.06	-0.15	-0.01	-0.00	1.00													
6	-0.16	0.06	0.30	-0.01	-0.52	1.00												
7	0.47	-0.23	-0.33	0.26	0.05	-0.17	1.00											
8	0.00	0.19	-0.03	0.09	-0.31	0.12	0.09	1.00										
9	-0.03	-0.16	-0.13	0.30	0.05	-0.21	0.10	0.45	1.00									
10	-0.12	0.10	-0.02	-0.01	-0.28	0.51	0.12	0.20	-0.26	1.00								
11	0.13	-0.30	-0.20	-0.05	0.27	-0.33	0.26	-0.30	0.16	-0.26	1.00							
12	0.50	-0.17	-0.34	-0.20	-0.13	-0.29	0.13	-0.04	0.06	-0.22	0.24	1.00						
13	-0.07	-0.06	0.12	-0.26	-0.05	0.05	-0.23	0.09	0.13	-0.13	-0.16	-0.00	1.00					
14	0.01	-0.24	-0.19	-0.19	0.04	0.05	0.01	-0.01	0.17	0.18	0.12	0.22	-0.09	1.00				
15	-0.15	0.02	-0.01	0.16	-0.26	0.18	0.02	0.07	0.19	0.40	-0.08	0.01	0.00	0.15	1.00			
16	-0.20	0.14	0.16	0.08	-0.18	0.21	0.03	0.11	-0.18	0.16	-0.20	-0.16	0.02	-0.20	0.09	1.00		
17	0.14	0.10	0.24	0.04	0.37	-0.03	-0.06	-0.16	0.18	-0.13	0.26	-0.02	0.22	0.11	-0.01	0.13	1.00	
18	-0.32	0.07	0.50	0.20	-0.04	0.06	-0.36	0.05	0.36	-0.09	0.03	-0.27	0.14	0.02	0.16	0.08	0.27	1.00

TABLE 8

FACTOR LOADINGS OF THE 18 DEPENDENT VARIABLES
AND SUM OF SQUARED LOADINGS FOR THE 7 OBTAINED FACTORS

Variable	I	II	III	IV	V	VI	VII	h^2
			Factor Loadings					
1	0.84	-0.13	-0.01	0.10	0.02	0.02	0.11	0.76
2	-0.05	-0.01	0.20	0.10	0.02	-0.08	0.84	0.77
3	-0.57	0.06	-0.06	0.33	0.05	-0.23	0.41	0.66
4	-0.11	-0.02	0.07	0.06	0.84	-0.14	0.04	0.76
5	-0.00	-0.37	-0.71	0.12	0.09	0.02	-0.01	0.67
6	-0.24	0.70	0.22	0.02	-0.17	-0.19	0.08	0.67
7	0.64	0.10	-0.08	-0.11	0.49	-0.20	-0.29	0.81
8	0.10	0.06	0.70	-0.00	0.14	-0.06	0.13	0.55
9	-0.12	-0.27	0.23	0.39	0.46	0.35	-0.40	0.78
10	-0.00	0.85	0.09	-0.16	0.04	-0.01	0.07	0.76
11	0.24	-0.19	-0.48	0.24	0.07	0.17	-0.38	0.55
12	0.66	-0.16	0.11	0.07	-0.27	0.32	-0.04	0.65
13	-0.13	-0.19	0.34	0.49	-0.49	-0.22	-0.31	0.78
14	0.11	0.36	-0.14	0.14	-0.12	0.70	-0.16	0.70
15	-0.06	0.53	0.26	0.20	0.26	0.26	-0.13	0.54
16	-0.05	0.32	0.08	0.19	0.03	-0.65	0.02	0.57
17	0.10	0.02	-0.37	0.84	-0.00	-0.07	0.15	0.87
18	-0.57	-0.06	0.12	0.55	0.21	0.16	0.56	0.72
Sum of Squared Loadings	2.41	2.06	1.77	1.72	1.67	1.47	1.45	

TABLE 9

FACTOR SCORES OF SUBJECTS ACCORDING TO EACH CONDITION (FOR STRAINS, 1 REFERS TO BALB/cJ, 2 TO ST/bJ; FOR SEX, 1 REFERS TO FEMALE, 2 TO MALE

			FACTOR						
Mother Strain	Pup Strain	Sex	I	II	III	IV	V	VI	VII
2	1	2	56.73	62.66	58.80	49.87	50.42	15.16	44.17
2	1	1	56.76	52.23	45.53	48.99	66.77	39.14	52.98
2	1	2	52.75	47.18	39.39	44.43	54.39	42.86	41.68
2	1	1	60.83	50.23	57.90	66.05	42.46	47.53	44.21
2	1	2	59.08	48.13	54.46	39.52	60.95	45.09	52.45
2	1	2	62.03	44.20	48.24	44.78	54.89	53.45	48.34
2	1	1	66.91	49.72	51.41	40.47	47.82	49.48	56.67
2	1	1	65.07	51.99	53.17	43.28	43.75	72.50	45.81
2	1	1	73.67	51.48	55.62	50.22	50.46	51.39	59.22
1	1	1	57.32	50.26	43.58	37.82	45.69	49.76	50.35
1	1	2	73.08	39.15	63.34	46.29	44.20	49.43	50.77
1	1	1	49.05	32.58	38.69	40.97	46.61	58.86	46.76
1	1	1	42.47	60.17	45.92	33.41	44.68	67.63	37.82
1	1	1	51.79	41.56	59.48	42.88	36.69	45.86	34.00
1	1	1	52.03	44.62	36.02	33.67	52.63	47.36	37.36
1	1	2	57.14	47.05	23.59	72.11	44.92	58.58	44.27
1	1	2	60.04	47.86	38.19	75.46	58.32	59.96	47.98
1	1	2	59.30	50.82	42.13	51.16	46.96	42.59	47.66
1	1	1	45.06	37.03	60.06	61.85	54.65	54.96	39.90
1	2	2	37.83	48.55	45.23	47.25	56.51	50.35	71.38
1	2	2	47.16	57.33	62.28	54.17	67.13	39.67	52.86
1	2	1	38.03	45.15	65.80	49.98	35.10	40.88	47.67
1	2	2	39.01	39.23	50.67	59.98	35.22	43.11	46.34
1	2	2	45.46	35.41	50.38	58.52	51.40	45.18	45.99
1	2	1	48.56	63.70	43.11	64.06	50.88	45.23	52.58
1	2	1	29.71	41.33	49.54	57.62	50.52	59.65	58.98
1	2	1	52.30	51.37	33.70	45.35	56.88	45.98	67.28
1	2	1	47.09	73.92	39.90	58.85	51.83	38.08	53.20
1	2	1	38.94	44.92	32.21	36.91	56.54	47.36	58.10
2	2	2	43.68	63.19	47.49	54.77	43.90	59.27	65.33
2	2	1	42.76	45.78	56.08	52.92	45.39	46.26	50.43
2	2	1	38.73	47.05	48.50	39.64	61.90	47.45	42.63
2	2	2	46.98	46.11	54.52	43.81	48.48	61.70	56.09
2	2	2	36.40	56.62	55.56	44.20	45.78	46.57	42.11
2	2	2	43.54	47.18	54.89	53.30	45.25	50.01	63.10
2	2	2	48.91	47.73	60.89	56.53	39.33	51.03	72.15
2	2	1	48.88	46.24	62.08	45.56	38.56	53.88	68.91
2	2	1	38.71	60.99	45.57	35.15	44.15	45.01	43.65
2	2	2	43.05	49.61	44.65	62.65	38.70	47.25	28.00
2	2	2	43.21	42.95	69.50	55.80	90.36	61.73	40.38
2	2	1	49.96	86.72	61.93	49.76	48.92	72.75	40.44

TABLE 10

RESULTS OF THE ANALYSIS OF VARIANCE
FOR FACTOR I

Source	Sum of Squares	d.f.	Mean Square	F
A	103.47	1	103.47	3.02
B	2364.79	1	2364.79	68.98**
C	19.08	1	19.08	0.56
A x B	35.51	1	35.51	1.04
A x C	241.66	1	241.66	7.05*
B x C	21.91	1	21.91	0.64
A x B x C	239.07	1	239.07	6.97*
ERROR	1131.30	33	34.28	

*$P<.05$
**$P<.01$

TABLE 11

RESULTS OF THE ANALYSIS OF VARIANCE
FOR FACTOR II

Source	Sum of Squares	d.f.	Mean Square	F
A	255.77	1	255.77	2.48
B	122.36	1	122.36	1.18
C	118.43	1	118.43	1.15
A x B	1.93	1	1.93	0.02
A x C	0.67	1	0.67	0.01
B x C	165.44	1	165.44	1.60
A x B x C	9.04	1	9.04	0.09
ERROR	3408.99	33	103.30	

TABLE 12

RESULTS OF THE ANALYSIS OF VARIANCE
FOR FACTOR III

Source	Sum of Squares	d.f.	Mean Square	F
A	476.96	1	476.96	4.92*
B	126.10	1	126.10	1.30
C	0.25	1	0.25	0.00
A x B	0.02	1	0.02	0.00
A x C	12.99	1	12.99	0.14
B x C	169.54	1	169.54	1.75
A x B x C	68.41	1	68.41	0.71
ERROR	3201.58	33	97.02	

*P<.05

TABLE 13

RESULTS OF THE ANALYSIS OF VARIANCE
FOR FACTOR IV

Source	Sum of Squares	d.f.	Mean Square	F
A	200.65	1	200.65	2.42
B	32.32	1	32.32	0.39
C	402.71	1	402.71	4.86*
A x B	0.53	1	0.53	0.01
A x C	224.09	1	224.09	2.70
B x C	5.84	1	5.84	0.07
A x B x C	560.52	1	560.52	6.76*
ERROR	2736.69	33	82.93	

*P<.05

TABLE 14

RESULTS OF THE ANALYSIS OF VARIANCE
FOR FACTOR V

Source	Sum of Squares	d.f.	Mean Square	F
A	16.46	1	16.46	0.14
B	0.00	1	0.00	0.00
C	80.37	1	80.37	0.68
A x B	134.79	1	134.79	1.15
A x C	6.84	1	6.84	0.06
B x C	2.31	1	2.31	0.02
A x B x C	5.30	1	5.30	0.05
ERROR	3872.48	33	117.35	

TABLE 15

RESULTS OF THE ANALYSIS OF VARIANCE
FOR FACTOR VI

Source	Sum of Squares	d.f.	Mean Square	F
A	0.28	1	0.28	0.00
B	0.00	1	0.00	0.00
C	139.32	1	139.32	1.47
A x B	621.71	1	621.71	6.57*
A x C	49.26	1	49.26	0.52
B x C	112.86	1	112.86	1.19
A x B x C	119.20	1	119.20	1.26
ERROR	3120.75	33	94.57	

*P<.05

TABLE 16

RESULTS OF THE ANALYSIS OF VARIANCE
FOR FACTOR VII

Source	Sum of Squares	d.f.	Mean Square	F
A	0.56	1	0.56	0.01
B	383.35	1	383.35	3.97
C	4.16	1	4.16	0.04
A x B	210.85	1	210.85	2.19
A x C	24.86	1	24.86	0.26
B x C	0.12	1	0.12	0.00
A x B x C	180.97	1	180.97	1.88
ERROR	3183.22	33	96.46	

TABLE 17

RESULTS OF THE ANALYSIS OF VARIANCE
FOR OFFSPRING WEIGHT AT 40 DAYS OF AGE

Source	Sum of Squares	d.f.	Mean Square	F
A	12.86	1	12.86	3.05
B	109.74	1	109.74	26.06**
C	73.61	1	73.61	17.48**
A x B	2.56	1	2.56	0.61
A x C	2.31	1	2.31	0.55
B x C	0.33	1	0.33	0.08
A x B x C	0.12	1	0.12	0.03
ERROR	143.15	34	4.21	

**P<.01

TABLE 18

CELL FREQUENCIES FOR THE 2 x 2 x 2
ANALYSIS OF VARIANCE ON OFFSPRING EMOTIONALITY

		Offspring Strain: BALB/cJ		Offspring Strain: ST/bJ	
		male	female	male	female
Post-Natal Mother Strain	BALB/cJ	4	6	4	6
	ST/bJ	4	5	7	5

FIGURE I

GRAPHICAL REPRESENTATION OF THE MEANS
FOR THE A x B x C INTERACTION FOR FACTOR I

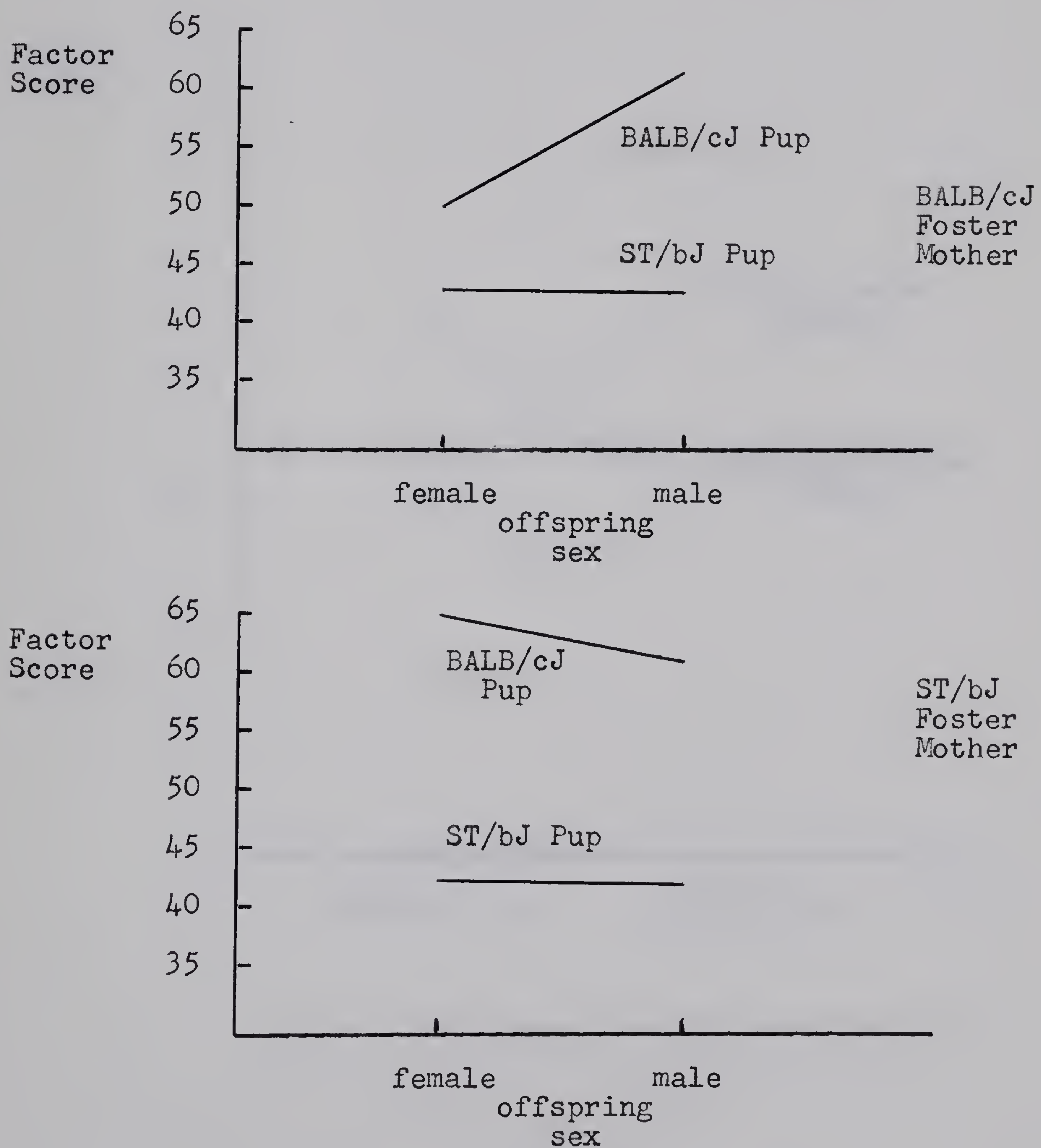
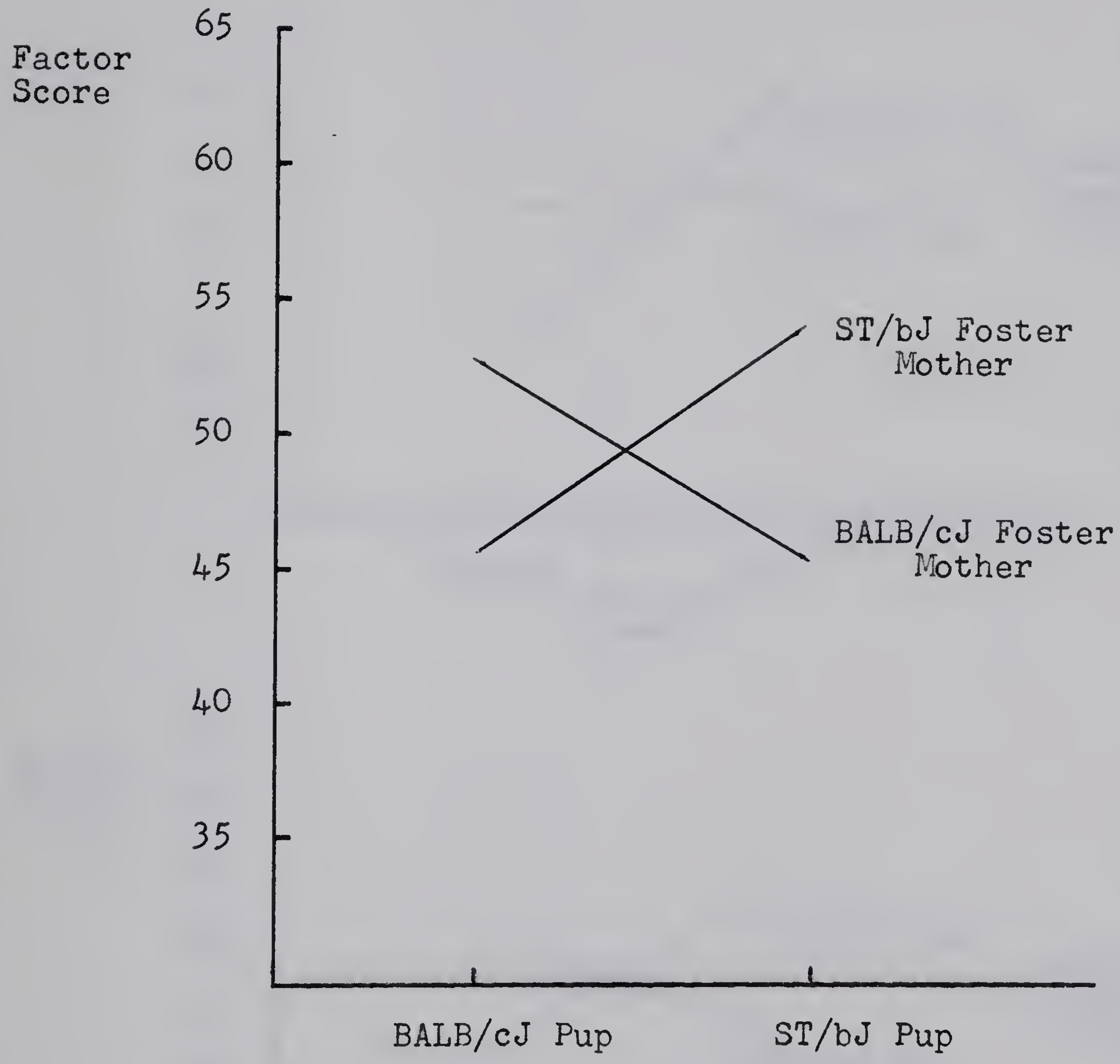


FIGURE 2

GRAPHICAL REPRESENTATION OF THE MEANS
FOR THE A x B INTERACTION FOR FACTOR VI



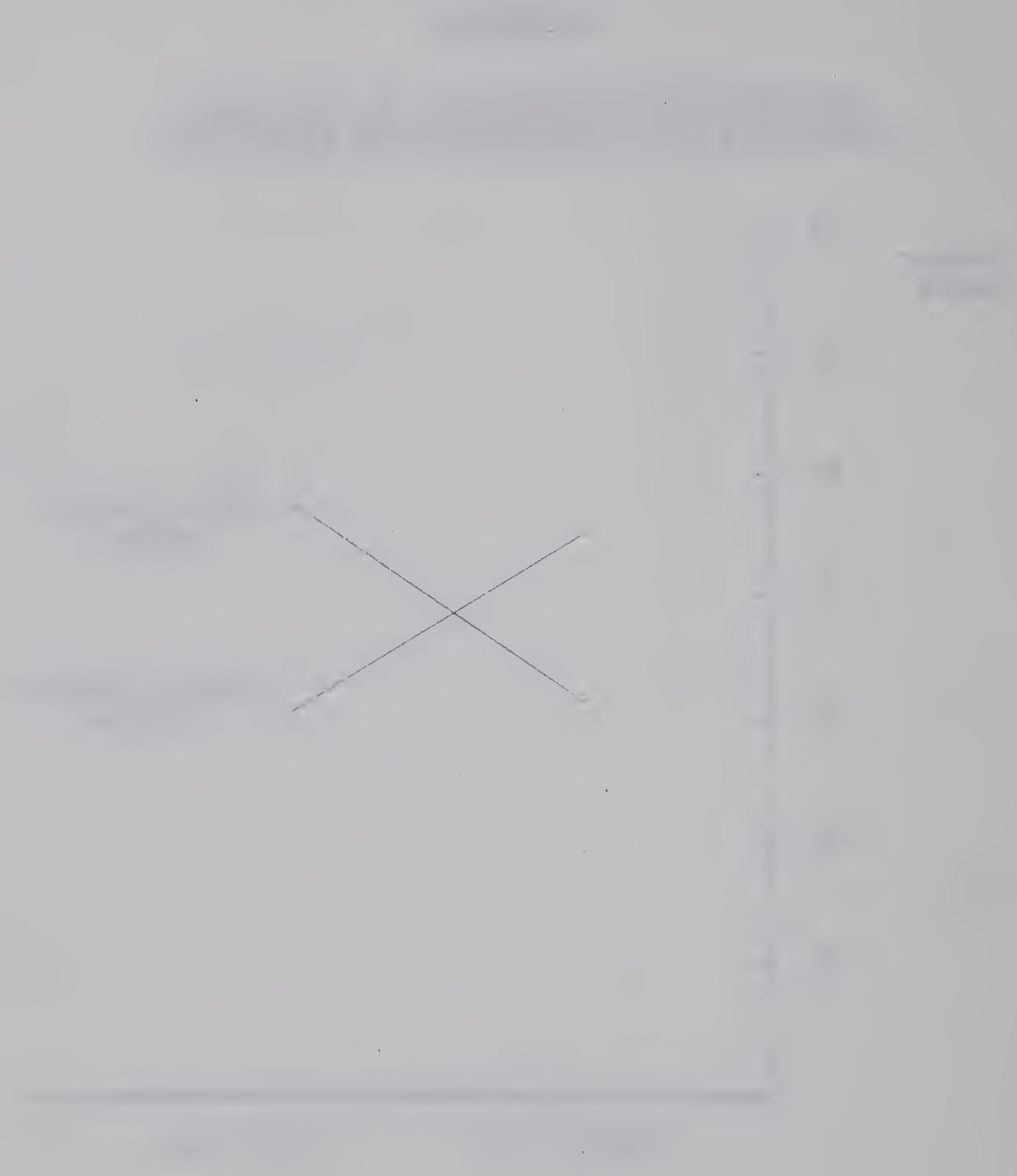


FIGURE 3

GRAPHICAL REPRESENTATION OF THE MEANS
FOR THE A x B x C INTERACTION FOR FACTOR IV

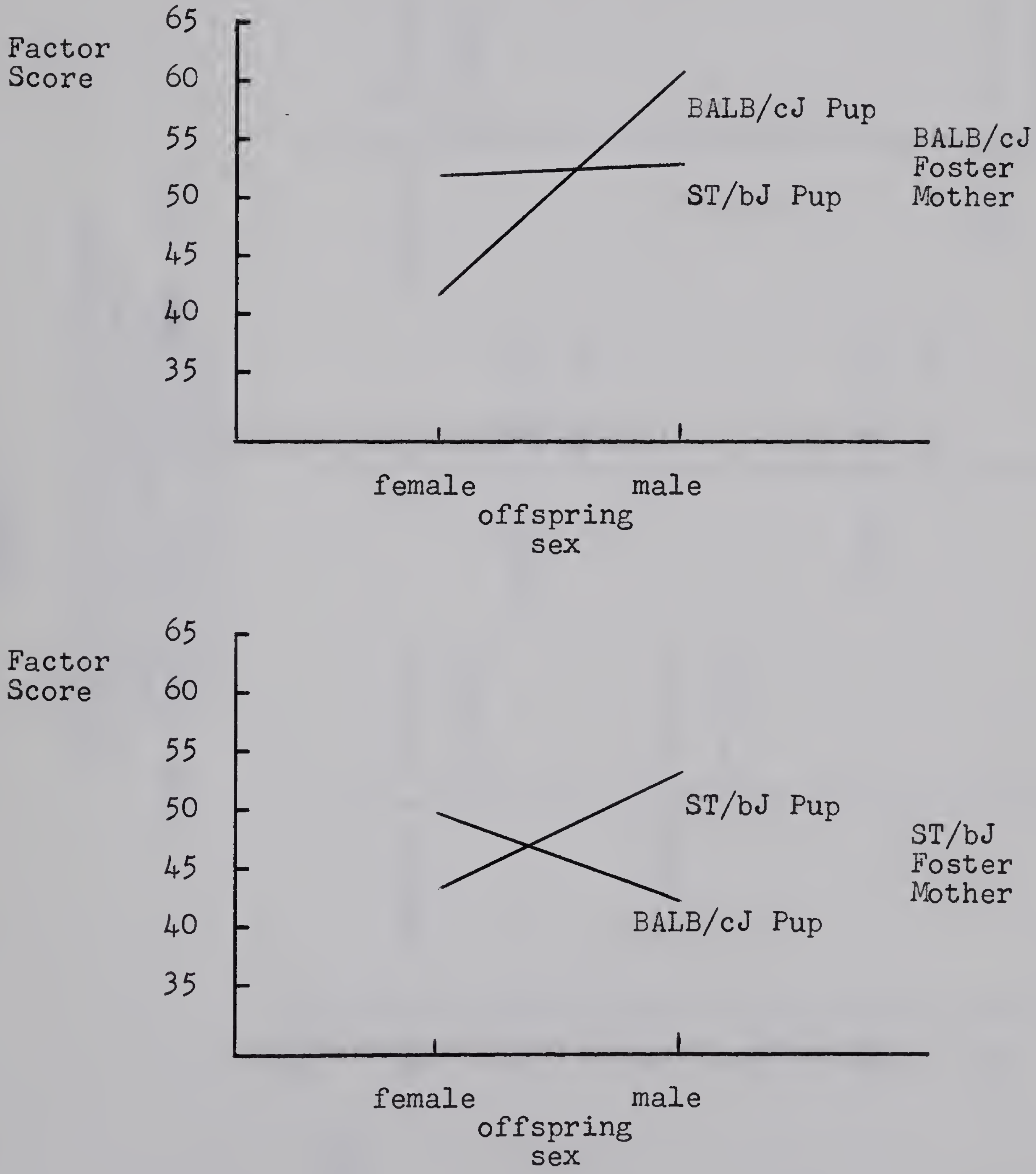


FIGURE 4

FACTOR SCORE MEANS AND VARIANCES
FOR THE BALB/cJ STRAIN (n=11)
AND ST/bJ STRAIN (n=11)
FROM ROYCE, CARRAN, AND HOWARTH (1967):
7TH OBLIQUE ROTATION